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Evaluation Report

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EVALUATION OF ECTOMYCORRHIZAE ON RED PINE SEEDLINGS

AT TOUMEY AND EVELETH NATIONAL FOREST NURSERIES

IN THE LAKE STATES

NORTHEASTERN AREA, STATE & PRIVATE FORESTRY
USDA FOREST SERVICE, UPPER DARBY, PA 19082

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Cover Photos:

Center: Mycorrhizal and Nonmycorrhizal red pine seedlings.
Lower Left: Mycorrhizal feeder root.
Lower Right: Nonmycorrhizal feeder root.

ABSTRACT: Three age classes of red pine seedlings were examined to determine what effects cultural practices at the USDA Forest Service nurseries in the Lake States had on ectomycorrhizae development. Development of ectomycorrhizae was very poor; trees were being shipped for planting with an average of 12 to 20 percent of the feeder roots with mycorrhizae. Nursery phosphorus (P_2O_5) levels seem to be too high for good mycorrhizae development. The percentage of mycorrhizal feeder roots on the seedlings increased as the cation exchange capacity decreased.

Ectomycorrhizae development in six red pine seedling beds (3 fumigated (MC-33) and 3 nonfumigated) at Toumey nursery was compared, but no differences were found.

Transplanted seedlings at the Toumey nursery had no more mycorrhizal feeder roots than transplanted seedlings.

INTRODUCTION

During January 1975, the Director of Timber Management, Region 9, requested that S&PF, FI&DM at St. Paul evaluate the mycorrhizae development on the red pine seedlings at Toumey (Watersmeet, Michigan) and Eveleth (Eveleth, Minnesota) Nurseries, and determine the need for increased percentages of mycorrhizal feeder roots on red pine. Mycorrhizal feeder roots are those resulting from the union of a beneficial fungus and a feeder root. Natural seeded 3-0 red pine seedlings near the two nurseries had between 65 and 100 percent of the feeder roots with mycorrhizae.

The evaluation was a cooperative project between State and Private Forestry, Forest Insect and Disease Management, North Central Forest Experiment Station, and the University of Minnesota, Department of Plant Pathology.

OBJECTIVES

The evaluation was carried out in three phases to determine:

A. The percentage of mycorrhizal feeder roots on 1-0[1/], 2-0 and 3-0 red pine seedlings, and the effects of cultural practices on the mycorrhizae.

B. The development of ectomycorrhizae on 2-0 and 3-0 red pine in fumigated and nonfumigated nursery beds.

C. The effects of transplanting on development of mycorrhizae on 1-3 red pine transplants at Toumey nursery.

A. Evaluation of the percentage of ectomycorrhizal feeder roots on 1-0, 2-0 and 3-0 red pine seedlings at Toumey and Eveleth Nurseries, and the effects of cultural practices on the mycorrhizae.

METHODS

Red pine (1-0, 2-0 and 3-0) were collected from Toumey and Eveleth Nurseries for mycorrhizal analysis in April, June, July and September. Two beds of each age class were sampled at each nursery. Forty seedlings were lifted from the middle rows of each bed. The seedlings were placed in plastic bags, cooled on ice, and taken to the laboratory at St. Paul, Minnesota, where the following data were recorded for each seedling:

1. Shoot height.
2. Fresh weight of shoot.
3. Dry weight of shoot.
4. Root length.
5. Fresh weight of root.
6. Dry weight of root.
7. Total number of feeder roots examined.
8. Number of feeder roots with mantles (The mantle is a fungus covering on the infected feeder roots).
9. Percentage of feeder roots with mantles that sho Hartig Net (Hartig Net is an intecullular network of the mycorrhizal fungus in the feeder root).

1/ (1-0) The first number is the years in the seedling bed and the second number is the years in a transplant beds.

Length measurements were recorded to the nearest millimeter and all weights to the nearest .01 gram. All feeder roots of the 1-0 and 2-0 seedlings were examined for ectomycorrhizal fungus mantle formation. The 3-0 stock was examined by stratifying the lateral roots by size, placing the roots end to end on a bench and selecting a 10 percent sample for analysis from the total length. The basis for classifying mycorrhizae was the presence of a mantle, usually accompanied by feeder root dichotomy. One mycorrhizal- and one nonmycorrhizal appearing feeder root, from each seedling was checked for indications of Hartig Net formation.

Soil samples were collected from each bed and analyzed for pH, electroconductivity, cation exchange capacity, N, P, K, type, and moisture holding capacity by the University of Minnesota. Nursery managers recorded the average monthly soil temperatures for each bed.

Data on the rate of soil fertilization, soil fumigation programs, and the use of pesticides were obtained for each sample bed.

Samples of suspected ectomycorrhizal sporophores were collected from the surrounding wind break trees and identified.

RESULTS AND DISCUSSION

The percentage of mycorrhizal feeder roots on the seedlings seems to be extremely low for all age classes in both nurseries (Tables 1 and 2). Only 5 percent of the 1-0 seedlings had at least one mycorrhizal feeder root. With the low number of feeder roots on the plant, the percentage of mycorrhizal feeder roots on the plant, the percentage of mycorrhizal feeder roots for all seedlings was increased when the percentages were averaged. An average of 95 percent of the 2-0 and 3-0 trees had mycorrhizae, but the percentage of mycorrhizal feeder roots at both nurseries was well below the desired 80 percent. The drop in the percentage of mycorrhizae present from September to the following year may be caused by root pruning. The percentage of mycorrhizal feeder roots should remain constant or increase with time in a given bed, if the seedlings are not root-pruned. The percentage of mycorrhizal feeder roots varied between beds.

An analysis of the relationship between the growth factors and percentage of seedlings with mycorrhizae was accomplished using simple regression analysis. No significant correlation was found between the percent mycorrhizal roots and the resulting seedling growth (Table 3). This lack of correlation may have been caused by the high fertility rates and lack of high percentages of mycorrhizae for comparison.

The percent of mycorrhizal roots was not correlated with soil fertility, fertilizer applications, pesticides used and monthly average soil temperatures. However, correlation was found between the percentage of mycorrhizal feeder roots and the cation exchange capacity ($r = -.57$). The phosphorus (P_2O_5 levels seem to be too high, in excess of 200 lbs/acre available phosphorus. The optimum range of available phosphorus for development of mycorrhizal fungus,

is estimated to be between 100 and 120 lbs/acre. The high soil phosphorus rates may suppress root development and, consequently, mycorrhizal fungus development. The negative correlation development of mycorrhizal roots and cation exchange capacity may indicate that increased exchangeable phosphorus inhibits development of mycorrhizae.

Table 1.--Mycorrhizal Feeder Roots on 1-0, 2-0 and 3-0 Red Pine Seedlings, at Four Different Lift Dates, Toumey Nursery, Watersmeet, Michigan, April - September, 1975

(in percent ± SE)

Seedling Age	Month April	June	July	Sept.
1-0	N/A[a/]	2.7±1.8	0 ± 0	1.1±1.3
2-0	2.0±1.6	2.0±1.6	1.0±1.1	25.6±4.9
3-0	8.6±3.2	9.5±3.3	11.5±3.6	20.2±4.5

[a/] N/A = Data not collected because germination had not occurred.

Table 2.--Mycorrhizal Feeder Roots on 1-0, 2-0 and 3-0 Red Pine Seedlings, at Four Different Lift Dates, Eveleth Nursery, Eveleth, Minnesota, April - September, 1975

(in percent ± SE)

Seedling Age	Month April	June	July	Sept.
1-0	N/A[a/]	1.4±1.3	0 ± 0	.5±1.2
2-0	1.4±1.3	1.1±1.2	8.7±3.2	17.6±4.3
3-0	1.2±1.2	1.6±1.4	14.3±3.9	12.9±3.8

[a/] N/A = Data not collected because germination had not occurred.

Table 3.--Height and Weight of 1-0, 2-0 and 3-0 Red Pine Seedlings for April, June, July and September at Toumey and Eveleth Nurseries, 1975[a/]

Seedling Age	Shoot Height (mm)				Fresh Weight (g)								Dry Weight (g)							
					Shoot				Root				Shoot				Root			
	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4	1	2	3	4
Toumey Nursery																				
1-0	N/A[b/]	41	27	60	N/A	.04	.21	1.7	N/A	.01	.09	.51		.01	.04	.31	N/A	.01	.02	.10
2-0	85	108	98	182	1.9	3.4	5.4	8.7	.91	.65	.98	1.93	.47	.65	1.20	2.53	.19	.13	.18	.48
3-0	129	171	234	233	5.3	6.6	14.2	12.7	1.79	1.88	2.92	2.19	1.43	1.76	3.94	3.88	.48	.54	.66	.59
Eveleth Nursery																				
1-0	N/A	39	34	103	N/A	.103	.19	.81	N/A	.01	.05	.10		.01	.04	.28	N/A	.00	.01	.09
2-0	50	71	123	147	.41	.85	2.3	3.3	.18	.22	.31	.89	.11	.17	.56	.89	.05	.05	.08	.22
3-0	54	145	248	195	2.4	5.0	9.2	10.2	.93	1.29	1.67	2.67	.66	1.22	2.15	3.00	.30	.37	.29	.56

[a/] Dates Lifted: 1 - April, 2 - June, 3 - July, 4 - September.

[b/] NA: Data not collected because germination had not occurred.

B. Evaluation of ectomycorrhizal development on red pine seedlings in fumigated and nonfumigated nursery beds in Michigan.

Materials and Methods

Roots of 2-0 and 3-0 red pine seedlings growing in fumigated (Mc-33)[2/] and unfumigated nursery beds were examined at the Toumey Nursery. The seedlings in each comparison bed were from the same seed source and sowed at about the same time.

Two seed sources for the 2-0 and one seed source for the 3-0 seedlings were examined for fumigation effects. Forty trees were lifted from 2 beds of each seed source. The total length of the bed was divided into 40 equal units, with one seedling lifted from each unit. The seedlings were then placed in cold storage before laboratory examination.

The shoot height of all seedlings was measured to the nearest millimeter. All mycorrhizal and nonmycorrhizal feeder roots were counted on the 2-0 seedlings. The roots of the 3-0 were stratified by size, placed end to end on a bench and a 10 percent sample was examined. The presence of mycorrhizae was confirmed by microscopic examination for mantle formation which was usually accompanied by feeder root dichotomy. One mycorrhizal and one nonmycorrhizal feeder root from each seedling was checked for Hartig Net formation.

Results

No significant difference in percentage of mycorrhizal feeder roots or development of mycorrhizal occurred between the fumigated and nonfumigated beds (Table 1). The shoot length on 3-0 stock and one bed comparison of the 2-0 stock revealed no difference. The other comparison of the 2-0 stock showed a 32mm increase height response in the fumigated beds when compared with the nonfumigated beds. The difference is significant at the 95 percent level of confidence (t - test).

Discussion

Apparently, fumigation of the red pine nursery beds with methyl bromide, containing 33 percent chloropicrin, was not a cause of the poor ectomycorrhizal fungus development. Since examination of windbreak trees adjacent to the sampled beds revealed an abundance of fruiting bodies of ectomycorrhizal fungi and all trees examined had some ectomycorrhizal feeder roots, it is concluded that other factors, such as soil fertility or biocides must have inhibited development of mycorrhizal fungi in the nursery beds.

As a follow-up, artificial introduction of Pisolithus tinctorius, a mycorrhizal fungus, and change of the fertilization schedule is planned to determine if the percentages of ectomycorrhizal roots can be increased.

[2/] Mc-33 = 76 percent Methyl Chloride and 33 percent Chloropicrin.

Table 1.--Seedling height and percentage of mycorrhizal feeder roots.

Seedling Age	Average Height (mm)				Percentage of mycorrhizal roots			
	Fumigated		Nonfumigated		Fumigated		Nonfumigated	
	Mean	SE	Mean	SE	Mean	SE	Mean	SE
1-0	80	± 8	76	± 4	2.9	± .5	1.6	± .5
2-0	94	± 7	62	± 4	2.1	± .5	1.6	± .4
3-0	202	± 14	197	± 15	13.9	± 3.	14.6	± 3.

C. Development of mycorrhizae on red pine transplants in the Toumey Nursery, 1975.

METHODS

Eighty trees were lifted at random from the 1-3 transplant beds at the Toumey Nursery, Watersmeet, Michigan, placed in cold storage and shipped to St. Paul for examination.

The roots were examined using the methods described in Part B.

RESULTS AND DISCUSSION

On the average, about 14 percent of feeder roots are mycorrhizal on the 1-3 red pine seedlings at Toumey. ($SE_{\bar{x}}$ = 2.8 percent; range 2-34 percent). The percentage of the mycorrhizal feeder roots on these transplants is similar to the percentages on the 3-0 red pine stock. Thus, the data shows that the percentage of mycorrhizal roots in normal nursery stock is not increased by transplanting. Since the transplants had not been fertilized or sprayed with pesticides, it seems that the initial soil fertility may be one of the major factors affecting mycorrhizal fungus development.

CONCLUSIONS

The following conclusions are based on results from the three phases of the evaluation.

1. The percentage of mycorrhizal feeder roots on the nursery seedlings is below the 70 to 95 percent found on natural seedlings of the same age.
2. No change in the percentage of mycorrhizal feeder roots may be achieved at Toumey through the elimination of fumigation.
3. Transplanting did not increase or decrease the percentage of mycorrhizal feeder roots.
4. The percentage of mycorrhizal feeder roots increased with seedling age in a given bed, but varied between beds.
5. No correlation was found between cultural practices and the percentage of the mycorrhizal feeder roots. This can be attributed to the consistantly low incidence of mycorrhizae and no variation in cultural practices.
6. The cation exchange capacity of the soil was correlated with the percentage of mycorrhizal feeder roots.
7. Over 200 lbs/acre phosphorus (P_2O_5) in the soil seems to present good mycorrhizal root development.
8. The percentage of mycorrhizal feeder roots was so low that no correlation between seedling size and mycorrhizal development was possible.

RECOMMENDATIONS

We recommend the following actions:

1. That S&PF determine mycorrhizal fungus development on other tree species at the Federal nurseries.
2. That S&PF cooperate with state agencies to evaluate mycorrhizae in state nurseries.
3. That S&PF introduce ectomycorrhizal fungi in forest nurseries to stimulate ectomycorrhizae development of, and increase growth rates and field survival.
4. That NCFES determine the optimum soil fertility needed to stimulate development of ectomycorrhizae in nursery-grown red pine.
5. That NCFES determine how to alter nursery culture practices to increase mycorrhizal fungus development.
6. That NCFES determine the optimum ectomycorrhizal development rate for nursery-grown red pine.
7. That NCFES develop improved methodology for inoculation of nursery beds with mycorrhizal fungi.
8. That NCFES determine if there is a more efficient species of fungus than *P. tinctorius* for inoculation of nursery-grown red pine.